UNCLASSIFIED
SECURITY CLASSIFICATION OF THIS PAGE

2

REPORT DOCUMENTATION PAGE

DTIC FILE COPY

Form Approved OMB No. 0704-0188

DTIC ELECTE NOV 16 1990 S D

AD-A228 540

1a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY 2b. DECLASSIFICATION / DOWNGRADING SCHEDULE	3. DISTRIBUTION / AVAILABILITY OF REPORT APPROVED FOR PUBLIC RELEASE DISTRIBUTION UNLIMITED
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S) AFOSR-TR- 90 1017
6a. NAME OF PERFORMING ORGANIZATION UNIVERSITY OF ILLINOIS COLLEGE OF VETERINARY MEDICINE	6b. OFFICE SYMBOL (*If applicable*)
6c. ADDRESS (*City, State, and ZIP Code*) DEPARTMENT OF VETERINARY BIOSCIENCES 2001 S. LINCOLN AVENUE URBANA, IL 61801	7a. NAME OF MONITORING ORGANIZATION AIR FORCE OFFICE OF SCIENTIFIC RESEARCH/NL 7b. ADDRESS (*City, State, and ZIP Code*) BUILDING 410 BOLLING AFB, D.C. 20332-6448
8a. NAME OF FUNDING / SPONSORING ORGANIZATION AFOSR	8b. OFFICE SYMBOL (*If applicable*) NL 9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER AFOSR 88-0033
8c. ADDRESS (*City, State, and ZIP Code*) BUILDING 410 BOLLING AFB, D.C. 20332-6448	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. 61102F; PROJECT NO. 2312; TASK NO A5; WORK UNIT ACCESSION NO.

11. TITLE (*Include Security Classification*)
A COMPARATIVE STUDY REGARDING THE ASSOCIATION OF ALPHA-2U GLOBULIN WITH THE NEPHROTOXIC MECHANISM OF CERTAIN PETROLEUM-BASED AIR FORCE FUELS

12. PERSONAL AUTHOR(S)
DR. THOMAS E. EURELL

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (*Year, Month, Day*)	15. PAGE COUNT
FINAL	FROM 12/1/87 TO 6/30/90	SEPTEMBER 17, 1990	13

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (*Continue on reverse if necessary and identify by block number*)
FIELD	GROUP	SUB-GROUP	ALPHA 2U GLOBULIN HYDROCARBON NEPHROTOXICITY LYSOSOMES

19. ABSTRACT (*Continue on reverse if necessary and identify by block number*)

Fisher 344 male rats have a dose and time-dependent renal proximal tubular degeneration induced by certain hydrocarbon compounds. We have used rat strain variation of the alpha-2U globulin molecule and metabolic alteration of the urinary pH as methods to investigate the hydrocarbon-induced nephrotoxic response. Three significant advances have been made during this project: (1) the development of a histochemical procedure to specifically evaluate decalin-induced changes in the lysosomes of rat renal tubular epithelial cells, (2) the discovery that pigmented male rats demonstrate hydrocarbon-induced nephrotoxicity, and (3) the discovery of a difference in the hydrocarbon-induced nephrotoxic response of male rats following alteration of the urinary pH. Sodium bicarbonate-induced elevation of the urinary pH markedly altered the lysosomal integrity and morphologic appearance of renal tubular cells in male rats exposed to decalin.

20. DISTRIBUTION / AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	UNCLASSIFIED
22a. NAME OF RESPONSIBLE INDIVIDUAL DR. T. JAN CERVENY	22b. TELEPHONE (*Include Area Code*) 202 767 5021 22c. OFFICE SYMBOL NL

DD Form 1473, JUN 86 *Previous editions are obsolete.*

SECURITY CLASSIFICATION OF THIS PAGE
UNCLASSIFIED

A COMPARATIVE STUDY REGARDING THE ASSOCIATION OF ALPHA-2U GLOBULIN WITH THE NEPHROTOXIC MECHANISM OF CERTAIN PETROLEUM-BASED AIR FORCE FUELS

AFOSR 88-0033

Final Technical Report (12/1/87-6/30/90)

Prepared by

Thomas E. Eurell, D.V.M., Ph.D.

of the

Department of Veterinary Biosciences

College of Veterinary Medicine

University of Illinois

September, 1990

Abstract

Fisher 344 male rats have a dose and time-dependent renal proximal tubular degeneration induced by certain hydrocarbon compounds. This degeneration may be associated with a low molecular weight urinary protein called alpha-2U globulin. We have used rat strain variation of the alpha-2U globulin molecule and metabolic alteration of the urinary pH as methods to investigate the hydrocarbon-induced nephrotoxic response. Three significant advances have been made during this project: (1) the development of a histochemical procedure to specifically evaluate decalin-induced changes in the lysosomes of rat renal tubular epithelial cells, (2) the discovery that pigmented male rats demonstrate hydrocarbon-induced nephrotoxicity, and (3) the discovery of a difference in the hydrocarbon-induced nephrotoxic response of male rats following alteration of the urinary pH. Sodium bicarbonate-induced elevation of the urinary pH markedly altered the lysosomal integrity and morphologic appearance of renal tubular cells in young, adult male rats exposed to decalin.

Introduction

The exposure of male rats to certain hydrocarbon compounds (e.g. decalin) results in a characteristic nephrotoxicity. The human renal response to hydrocarbon exposure is less clearly defined. Epidemiologic studies have failed to demonstrate any consistent association between long term, low level human hydrocarbon exposure and renal disease. However, the potential human health hazard of environmental or occupational hydrocarbon exposure has warranted continued epidemiological and mechanistic studies.

Although the primary animal model for human risk assessment of hydrocarbon compounds is the rat, there is considerable controversy regarding the validity of this model. The basis for the controversy is centered on a urinary protein called alpha-2U globulin (A2U), which appears to be unique to the rat. The principal investigator, in collaboration with toxicologists at AAMRL/THT, Wright-Patterson AFB, is studying the association of A2U with the hydrocarbon-induced nephrotoxic process.

We have shown that the minor A2U isoelectric variants of albino rat strains can differ from those of the pigmented strains (Eurell et al., 1988). Therefore, we suspected that albino (Fisher 344 and Sprague-Dawley) male rats might show a different decalin-induced nephrotoxicity than pigmented (Long-Evans and Fawn-Hooded) male rats. We were also interested in how metabolic alteration of the renal tubular cell environment might affect the development of hydrocarbon nephrotoxicity.

The principal objectives of this study were:

(I) To evaluate the relative sensitivities of albino and pigmented rat strains to hydrocarbon-induced nephrotoxicity.

(II) To evaluate hydrocarbon-induced nephrotoxicity after genetic modification of A2U by interstrain breeding.

(III) To compare metabolic alteration of urinary pH with the occurrence of nephrotoxicity.

This is the final report for the project (AFOSR-88-0033) which represents our efforts from December 1, 1987 through June 30, 1990.

Accomplishments

Objective (I)-Relative sensitivities of rat strains

Urinary A2U. We confirmed our previous findings (Eurell et al., 1988) regarding the molecular heterogeneity and concentration of urinary A2U from different rat strains. We noted an increased concentration of urinary A2U in all experimental animals (relative to control animals). Electrophoretic analysis of the A2U molecule revealed minor differences

in the A2U isoelectric variants between the different strains of experimental animals following decalin exposure. However, the pI=5.4 and 5.5 isoelectric variants (Figure 1) were the major A2U components seen following decalin-exposure in both pigmented and non-pigmented animals. Of all the strains studied, the Fisher 344 male rat had the highest concentration (judged by electrophoretic band density) of the pI=5.4 and 5.5 A2U isoelectric variants.

Histopathology. Hematoxylin and Eosin stained kidney sections revealed a marginal difference in the lysosomal accumulation of renal tubular epithelial cells between the albino and pigmented rat strains (Tables 1-3). The Fawn/Hooded rat appeared to be less susceptible to the nephrotoxicity than either the Fisher 344 or the Long/Evans strain. However, we believed the standard Hematoxylin and Eosin staining technique was not sufficiently sensitive nor specific to reliably detect subtle strain differences in the decalin-induced nephrotoxic response. Therefore, to increase the sensitivity and specificity of our histopathologic analysis of lysosomal changes in renal cells, we developed a naphthol AS-TR phosphate-pararosaniline (NPP) stain for acid phosphatase (a lysosomal marker). In addition, quantitative histochemical techniques were developed to verify the morphologic data (Eurell, et al., 1989; Eurell et al., 1990a).

The NPP procedure revealed striking differences between the lysosomes of control and experimental animals (Figures 2 and 3). However, there were no significant lysosomal differences detected between experimental animals from pigmented and non-pigmented rat strains using the NPP stain. We believe that the NPP procedure is more reliable than Hematoxylin and Eosin staining for comparison of the strain susceptibility to decalin nephrotoxicity. Therefore, we conclude that there was no significant difference between the decalin-induced nephrotoxic response of the pigmented and non-pigmented strains used in this study.

We are currently preparing a manuscript to describe the nephrotoxic response of different rat strains following decalin exposure (Eurell, et al., 1990b). These findings are important as they support the hypothesis that hydrocarbon-induced, male rat nephropathy is not strictly limited to albino rats. Furthermore, if decalin-induced nephrotoxicity is associated with A2U, it most likely involves the major A2U isoelectric variants (pI=5.4 and 5.5).

Objective (II)-Results of interstrain breeding on nephrotoxic response

Young, adult F1 male rats from interstrain breedings (Fawn/Hooded male x Fisher 344 female and Long/Evans male x Fisher 344 female) were exposed to decalin and their nephrotoxic response compared to that of the parental strains (Tables 4 and 5). The major A2U isoelectric variants of the F1 male rats were the same as their parental strains (pI=5.4 and 5.5). There was no significant difference detected between the nephrotoxic response of the F1 male rats and the parental strains. These data further support the findings obtained under objective I.

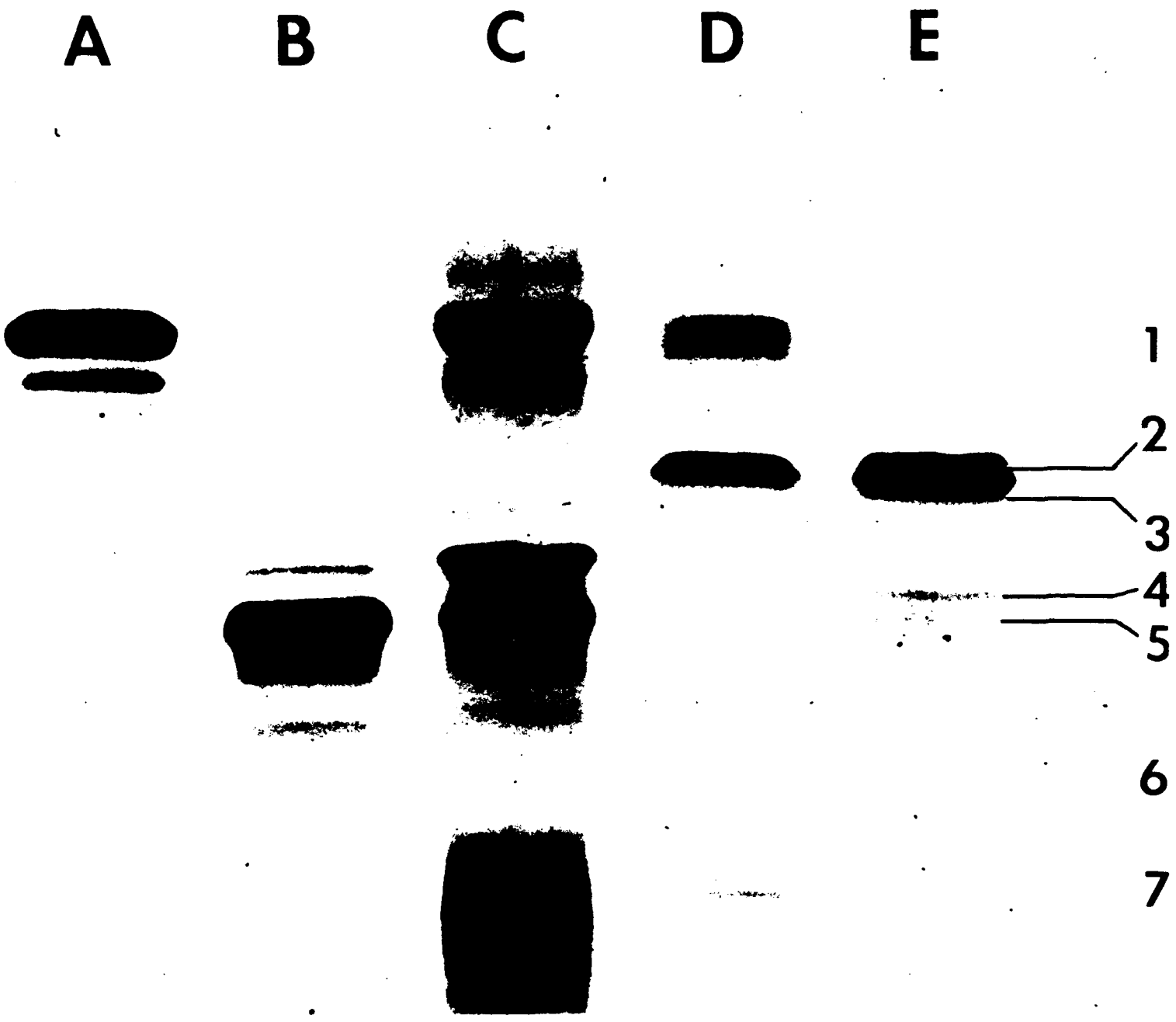

Figure 1. Isoelectric focusing pattern of urinary A2U from Fisher 344 male rat. Columns A, B, and C are protein standards and column D is a crude A2U extract used to calibrate the isoelectric focusing gel. The isoelectric variants of purified A2U are shown in column E and have the following pI values: (1)=6.0, (2)=5.5,(3)=5.4, (4)=5.3, and (5)=5.1

Table 1. Pathology summary sheet following decalin exposure.

Fisher 344 Male Rat

TREATMENT	HYALINE DROPLETS IN PROX. EPI.	CORTICAL TUBULAR DILATION	PROXIMAL TUBULAR EPI. NECROSIS	CASTS	OTHER
H2O (2.0 ML/KG)					
88-1	+/-	+/-	-	-	-
88-2	+/-	+/-	-	-	-
88-3	+/-	+/-	1	-	-
88-4	+/-	+/-	-	-	-
DECALIN (1.0 ML/KG)					
88-5	2	1	1	OCC. (H)	#2
88-6	2	1	+/-	-	-
88-7	2	+/-	1	-	#1
88-8	2	+/-	1	-	#1
DECALIN (2.0 ML/KG)					
88-9	2-3	1	1-2	1 (H/C)	#1
88-10	2	1	1-2	1 (H/C)	#1
88-11	3	+/-	2	1 (H/C)	#1,#3
88-12	2	+/-	1	-	#1

NOTE:OCC.=OCCASIONAL;(H)=HYALINE;(H/C)=HYALINE/CELLULAR; #1=PROXIMAL TUBULAR EPI.REGENERATION; #2=FOCAL PERIVASCULAR LYMPHOID AGGREGATES; #3=CORTICAL TUBULAR NEPHROLITHS (BASOPHILIC OVOID BODIES)

SCORING- (+/-)=MINIMAL; (1)=MILD; (2)=MODERATE; (3)=SEVERE

Table 2. Pathology summary sheet following decalin exposure.

Long/Evans Male Rat

TREATMENT	HYALINE DROPLETS IN PROX. EPI.	CORTICAL TUBULAR DILATION	PROXIMAL TUBULAR EPI. NECROSIS	CASTS	OTHER
H2O (2.0 ML/KG)					
88-13	+/-	+/-	-	-	-
88-14	+/-	-	-	-	-
88-15	+/-	+/-	-	-	-
88-16	+/-	-	-	-	-
DECALIN (1.0 ML/KG)					
88-17	2	+/-	1	-	#1
88-18	2-3	+/-	+/-	OCC. (H)	#1
88-19	2	1	1	-	#1
88-20	2	+/-	1	OCC. (H/C)	#1,#2
DECALIN (2.0 ML/KG)					
88-21	2-3	2	1-2	2 (H/C)	#1,#2
88-22	2	1	1-2	1 (H/C)	#1
88-23	2	1	2	1 (H/C)	#1
88-24	2-3	1	2	2 (H)	#1

NOTE:OCC.=OCCASIONAL;(H)=HYALINE;(H/C)=HYALINE/CELLULAR; #1=PROXIMAL TUBULAR EPI.REGENERATION; #2=FOCAL PERIVASCULAR LYMPHOID AGGREGATES.

SCORING- (+/-)=MINIMAL; (1)=MILD; (2)=MODERATE; (3)=SEVERE

Table 3. Pathology summary sheet following decalin exposure.

Fawn/Hooded Male Rat

TREATMENT	HYALINE DROPLETS IN PROX. EPI.	CORTICAL TUBULAR DILATION	PROXIMAL TUBULAR EPI. NECROSIS	CASTS	OTHER
H2O (2.0 ML/KG)					
88-25	+/-	-	-	-	-
88-26	1	-	-	-	-
88-27	+/-	+/-	-	-	-
88-28	+/-	+/-	-	-	-
DECALIN (1.0 ML/KG)					
88-29	1	+/-	+/-	-	#1
88-30	1-2	-	+/-	-	#1
88-31	2	1	1	OCC. (H/C)	#1,#2
88-32	1-2	+/-	1	-	#1
DECALIN (2.0 ML/KG)					
88-33	1-2	1	1-2	-	#1,#2
88-34	2	1	1	OCC. (H/C)	#1
88-35	2	1	2	-	#1
88-36	2	+/-	2	-	#1,#2

NOTE:OCC.=OCCASIONAL;(H/C)=HYALINE/CELLULAR; #1=PROXIMAL TUBULAR EPI. REGENERATION; #2=FOCAL PERIVASCULAR LYMPHOID AGGREGATES.

SCORING- (+/-)=MINIMAL; (1)=MILD; (2)=MODERATE; (3)=SEVERE

Table 4. Pathology summary sheet following decalin exposure.

F1 Male Rat From Long/Evans Male x Fisher 344 Female Breedings

TREATMENT	HYALINE DROPLETS IN PROX. EPI.	CORTICAL TUBULAR DILATION	PROXIMAL TUBULAR EPI. NECROSIS	CASTS	OTHER
H2O (2.0 ML/KG)					
89-1	+/-	+/-	-	-	-
89-2	+/-	-	-	-	-
89-3	+/-	-	-	-	-
DECALIN (2.0 ML/KG)					
89-4	3	1	1	-	#1,#2
89-5	2	1	+/-	-	-
89-6	2	+/-	1	-	#1
89-7	2	+/-	1	-	#1
89-8	2-3	1	1-2	-	#1
89-9	2	1	1-2	-	#1
89-10	2-3	+/-	2	-	#1
89-11	2	+/-	1	-	#1
89-12	3	2	1-2	-	#1,#2

NOTE:#1=PROXIMAL TUBULAR EPI.REGENERATION;#2=FOCAL PERIVASCULAR LYMPHOID AGGREGATES

SCORING- (+/-)=MINIMAL; (1)=MILD; (2)=MODERATE; (3)=SEVERE

Table 5. Pathology summary sheet following decalin exposure.

F1 Male Rat From Fawn/Hooded Male x Fisher 344 Female Breedings

TREATMENT	HYALINE DROPLETS IN PROX. EPI.	CORTICAL TUBULAR DILATION	PROXIMAL TUBULAR EPI. NECROSIS	CASTS	OTHER
H2O (2.0 ML/KG)					
89-13	1	-	-	-	-
89-14	+/-	-	-	-	-
89-15	+/-	-	-	-	-
DECALIN (2.0 ML/KG)					
89-16	2	+/-	1	-	#1
89-17	2-3	+/-	+/-	-	#1
89-18	2	1	1	-	#1
89-19	2	+/-	1	-	#1,#2
89-20	2-3	2	1-2	-	#1,#2
89-21	2	1	1-2	1 (H)	#1
89-22	2	1	2	1 (H/C)	#1
89-23	2-3	1	2	-	#1
89-24	3	2	1	-	#1,#2

NOTE:(H)=HYALINE;(H/C)=HYALINE/CELLULAR; #1=PROXIMAL TUBULAR EPI. REGENERATION; #2=FOCAL PERIVASCULAR LYMPHOID AGGREGATES.

SCORING- (+/-)=MINIMAL; (1)=MILD; (2)=MODERATE; (3)=SEVERE

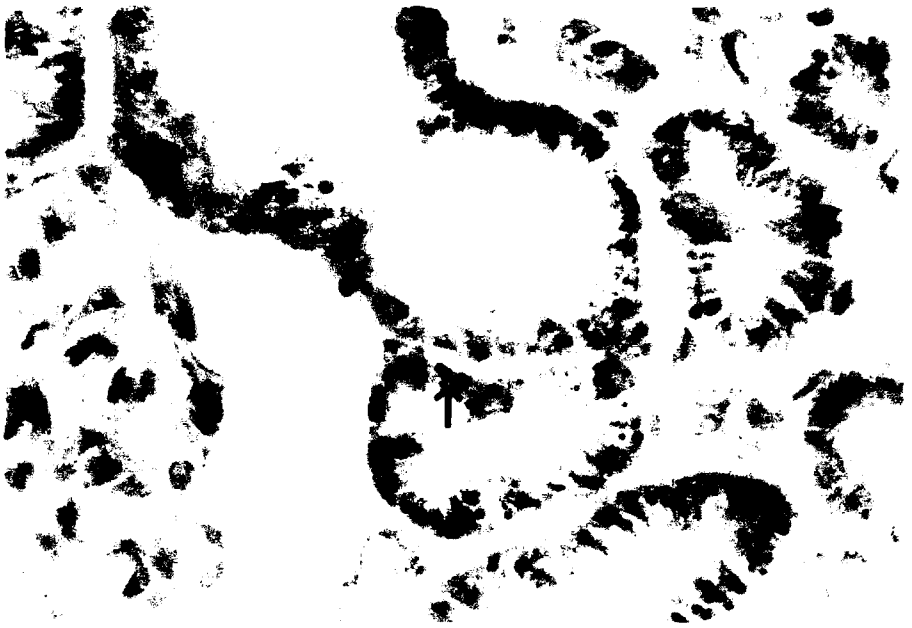

Figure 2. Naphthol AS-TR phosphate-pararosaniline stain for lysosomal acid phosphatase. Control Fisher 344 male rat given 2 ml/kg H_2O by gavage. Note small, dense, reddish-staining lysosomes in renal tubular epithelial cells (→). Methyl green counterstain, magnification x 1000.

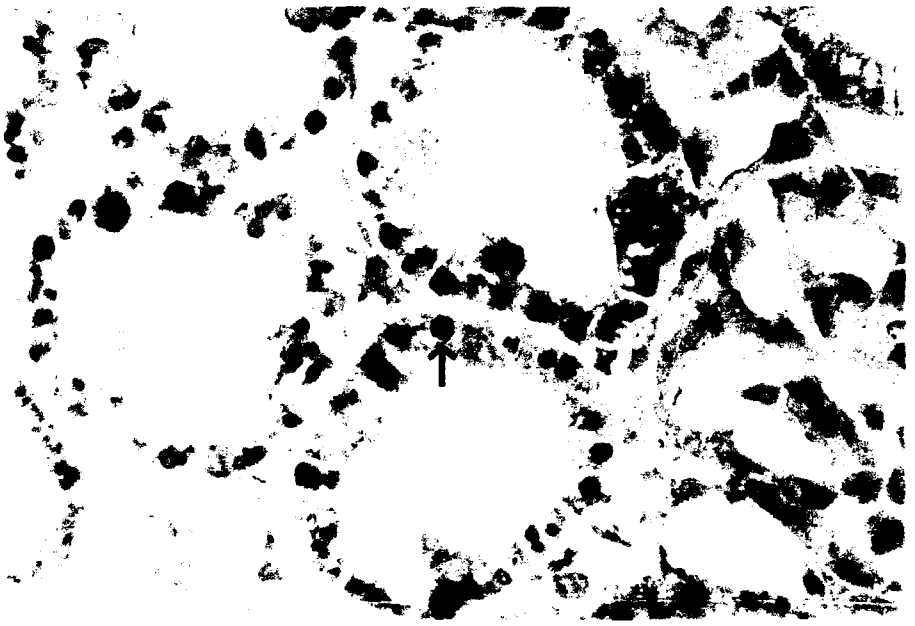

Figure 3. Naphthol AS-TR phosphate-pararosaniline stain for lysosomal acid phosphatase. Experimental Fisher 344 male rat given 2 ml/kg decalin by gavage. Note large, swollen, pale orange lysosomes in renal tubular epithelial cells (→). Methyl green counterstain, magnification x 1000.

Objective (III)-Metabolic alteration of urinary pH

Experimental animals were dosed with either 6% sodium bicarbonate or 6% ammonium chloride (1 ml/100 gm) by gavage. Using this procedure, the urine of experimental animals was significantly altered (mean pH of ammonium chloride treated animals=5.5; mean pH of sodium bicarbonate treated animals=8.2). We found the gavage procedure to be much more reliable than previously published methods (e.g., chemicals added to drinking water). Half the animals were also treated with decalin (2 ml/kg, 1 hr following either sodium bicarbonate or ammonium chloride treatment.

The sodium bicarbonate, ammonium chloride, and ammonium chloride/decalin treatment had no significant effect on the NPP stain reaction of tubular epithelial cells. However, the sodium bicarbonate/decalin treatment had a marked effect on the NPP stain reaction of tubular epithelial cells related to lysosomal lysis (Figure 4). The lysosomal lysis associated with decalin exposure under conditions of urinary alkalosis is a unique feature of the nephrotoxicity associated with the combined effect of the cellular pH and hydrocarbon agent. This finding is important because it may provide one of the first breakthroughs in understanding the mechanism of hydrocarbon-induced nephrotoxicity. We are currently preparing a manuscript to describe the effect of urinary pH on male rat nephropathy (Eurell, et al 1990c).

This finding takes on particular importance in relation to an Air Force fuel (JP-10) because of a recent finding by the principal investigator in collaboration with Dr. David Mattie (AAMRL/THT). Exposure of male rats to JP-10 (1.5 ml/kg) induces lysosomal lysis in renal tubular cells analogous to the combined effect of sodium bicarbonate/decalin exposure (Figure 5). The lysosomal lysis effect of JP-10 exposure is different than the effects of any previously reported nephropathy inducing agents (e.g., decalin, trimethylpentane, limonene, unleaded gasoline) and may provide the basis for an effective structure-activity relationship analysis.

Summary

The new techniques and insight gained through this research project have advanced the methods available to study male rat nephropathy and perhaps provided one of the first systems to effectively approach a structure-activity relationship analysis. The principal investigator, in collaboration with Dr. David Mattie and other colleagues at AAMRL/THT, plans to extend these investigations by comparing: (1) the nephrotoxic response of Fisher 344 and NBR male rats and (2) the relative nephrotoxicities of JP-10, JP-4, and JP-8 fuels.

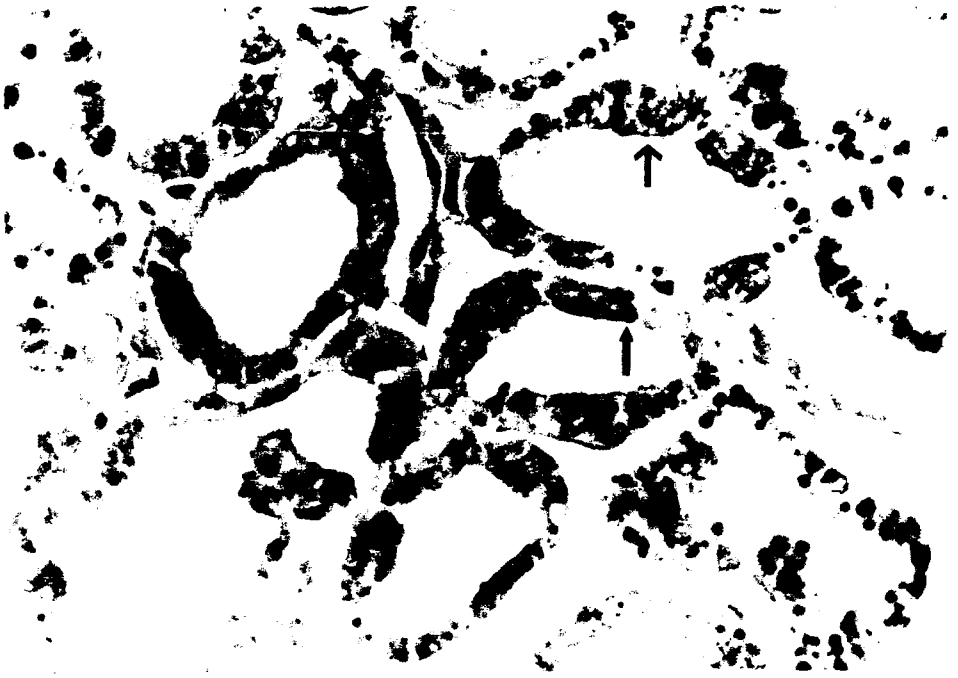

Figure 4. Naphthol AS-TR phosphate-pararosaniline stain for lysosomal acid phosphatase. Experimental Fisher 344 male rat given a combination of sodium bicarbonate (1ml/kg)/decalin (2ml/kg). Note enlarged, intact lysosomes (➞) and areas of lysosomal lysis (→). Methyl green counterstain, magnification x 500.

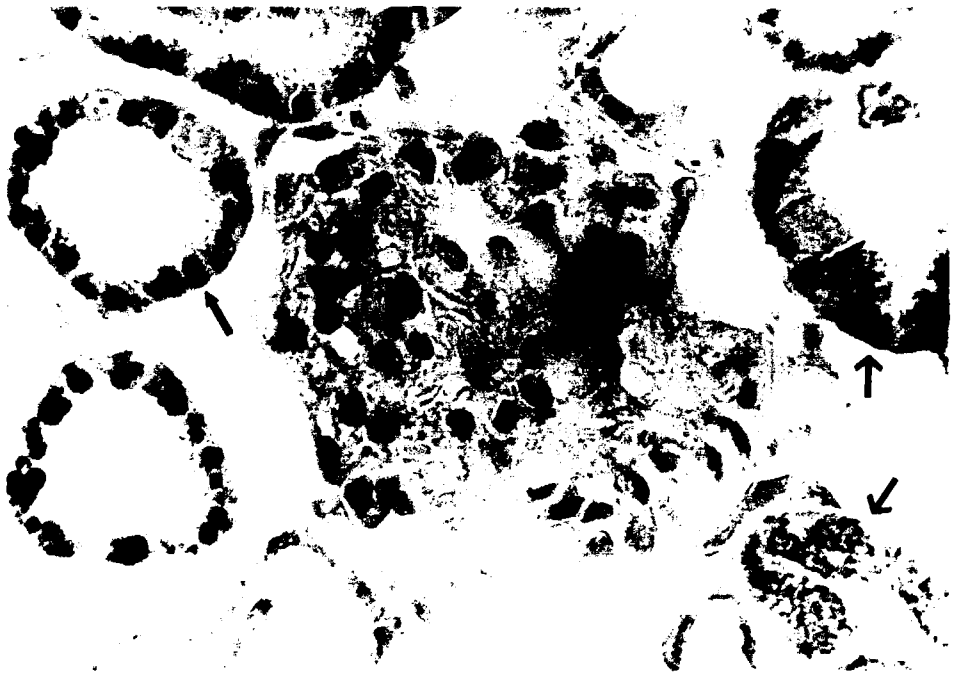

Figure 5. Naphthol AS-TR phosphate-pararosaniline stain for lysosomal acid phosphatase. Experimental Fisher 344 male rat given JP-10 (1.5 ml/kg). Note enlarged, intact lysosomes (➞) and areas of lysosomal lysis (→). Methyl green counterstain, magnification x 800.

Publications

Eurell, T.E., Parnell, M.J., and Henningsen, G.M. Comparison of A2U globulin isolated from the urine of albino and non-albino male rats. The Toxicologist, (8:1):536, 1988.

Eurell, T.E., Parker, R.D., and Alden, C.L. Lysosomal changes in renal tubular epithelial cells of male Sprague-Dawley rats following decalin exposure. The Toxicologist, (9:1):80, 1989.

Eurell, T.E., Eurell, J.C., Schaeffer, D.J., Mattie, D.R., and Alden, C.L. Lysosomal changes in renal proximal tubular epithelial cells of male Sprague-Dawley rats following decalin exposure. Tox. Path. (accepted for publication) Volume 18(4), 1990.

Eurell, T.E., Parnell, M.J., Henningsen, G.H., and Mattie, D.R. Nephrotoxic effect of decalin exposure on pigmented and albino male rats. (in preparation).

Eurell, T.E., Mattie, D.R., and Alden, C.L. The effect of urinary pH on the nephrotoxicity of decalin in Fisher 344 male rats. (in preparation).

Interactions (Coupling Activities)

Dr. Eurell conferred with Dr. David Mattie and Ms. Marilyn George at AAMRL/THT, Wright-Patterson AFB on 11/02/88 and 8/25/89. During the visits Dr. Eurell presented an update on the project and planned the next set of experiments in collaboration with AAMRL/THT staff.

Dr. Myrtle Davis obtained a M.S. in Veterinary Science while working on a component of this project under the direction of Dr. Eurell. The title of her thesis was "Immunocytochemical Localization of Alpha 2U-Globulin in the Proximal Tubules of Male Rats Following Decalin Exposure".